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Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 3.^{1,2} Aromatic Amine Analogues

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A series of artemisinin (1) derivatives containing bromo and heterocyclic or aromatic amine functions was prepared in the search for analogues with good water solubility and high antimalarial activity. Treatment of dihydroartemisinin (2a) with boron trifluoride etherate at room temperature gave the key intermediate, 9,10-dehydrodihydroartemisinin (3), which, on reaction with bromine, gave the dibromide 4. The latter was condensed with amines in anhydrous CH_2Cl_2 at $< 10^\circ\text{C}$ to give the desired products in 25–55% yield. The new derivatives, tested in vitro against *Plasmodium falciparum*, were found to be more effective against W-2 than D-6 clones and were not cross-resistant with existing antimalarials. Compound 6b, 3-fluoroaniline derivative, was the most active of the series, with the $\text{IC}_{50} \leq 0.16 \text{ ng/mL}$, making it several fold more potent than 1. However, no significant in vivo antimalarial activity against *Plasmodium berghei* was observed in any of the new compounds tested.

Artemisinin (qinghaosu, arteannuin, 1), an antimalarial agent isolated from the plant *Artemisia annua*, is an endoperoxide-containing sesquiterpene lactone.^{3–7} The unusual chemical structure of artemisinin coupled with its low toxicity and proven antimalarial efficacy have attracted attention from both chemists and parasitologists since its discovery in 1972. The practical use of artemisinin as an antimalarial agent, however, is impaired by (a) its insolubility in both water and oil,⁸ (b) its poor efficacy by oral administration,⁹ and (c) the rate of recrudescence in treated patients.⁹

The lactol form of 1, dihydroartemisinin (2a), prepared by the sodium borohydride reduction of the parent com-

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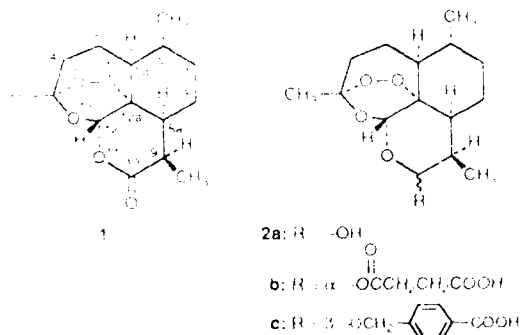
Table 1. Physical Properties of 9-Bromo-10-aminated Dihydroartemisinin Derivatives

compd	mp, °C	recrystn solvent	TLC ^a solvent (v/v)	% yield
6a	162–164	hexane + EtOAc	hexane–EtOAc (1:1)	30
7a	154–156	hexane + EtOAc	hexane–EtOAc (1:2)	10
6b	141–142	hexane + EtOAc	hexane–EtOAc (5:1)	25
7b	137–139	hexane + EtOAc	hexane–EtOAc (5:1)	10
6c	151–152	hexane + EtOAc	hexane–EtOAc (5:1)	35
6d	155–157	acetone + hexane	hexane–EtOAc (1:3)	36
6e	165–166	acetone + hexane	5% MeOH–CHCl ₃	51

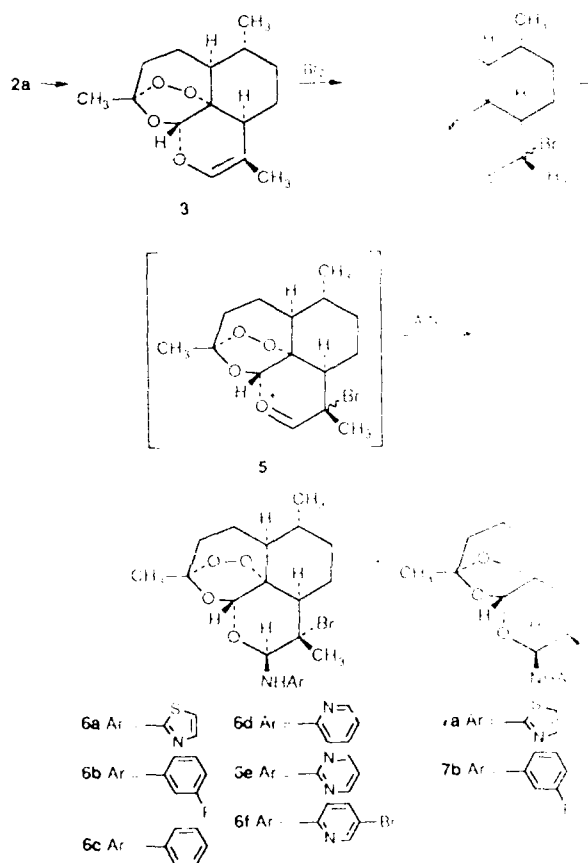
compound, was shown to be more active than 1.⁴⁸ But, because of its hemiacetal structure, the compound is believed to have lower stability than 1.

Chemical modifications of dihydroartemisinin to improve its efficacy and solubility have been reported by several laboratories. Artemether^{9,12} and arteether,^{9,10,12} the methyl and ethyl ethers of **2a**, respectively, are more lipophilic and more effective than artemisinin. Sodium artesunate (**2b**), a water-soluble derivative of **2a**, was demonstrated to be particularly useful in the treatment of cerebral malaria.^{9,10,12} A half ester of succinic acid, sodium artesunate is, however, not stable in aqueous solution, a property that is detrimental to its practical utility as an antimalarial agent.¹

Recently, we prepared a series of derivatives of dihydroartemisinin (**2a**) in which the solubilizing group, carboxylate, was coupled to dihydroartemisinin by an alkyl ether rather than an ester linkage.^{1,2} Among the water-soluble derivatives that we prepared, the sodium salt of artemic acid (**2c**) was found to be equally active as the parent artemisinin and sodium artesunate (**2b**) in vitro and more active than 1 and **2b** in rodent *Plasmodium berghei* test systems.¹ Furthermore, sodium artemic acid is sub-



stantially more stable than sodium artesunate in weakly alkaline aqueous solution, an important physical property for the preparation of an intravenous injection dosage form. Antimalarial studies have shown that sodium artemic acid totally eliminated the parasitemia in mice infected with *P. berghei* when administered in their drinking water.¹⁴

Scheme 1

In our continuing search for new artemisinin analogues with good water solubility and high antimalarial efficacy, we report here the preparation and antimalarial studies of additional dihydroartemisinin derivatives which contain bromo and heterocyclic or aromatic amine functions. Water solubility, it was anticipated, would be achieved through salt formation.

Chemistry

The starting material, dihydroartemisinin (**2a**), was prepared by sodium borohydride reduction of **1** according to a modified literature procedure.² Experiments have shown that proper adjustment of the pH of the reaction mixture before workup is critical to obtaining acceptable yields of product.

Dihydroartemisinin, upon treatment with boron trifluoride etherate at room temperature, gave a key intermediate, 9,10-dehydrodihydroartemisinin (**3**) in 75–80% yield (Scheme 1). Compound **3** was reported earlier to be a minor product when dihydroartemisinin was treated with alcohols under the boron trifluoride etherate catalysis and is probably formed by tautomerization of the oxonium intermediate, as discussed in the previous report.²

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Table I. ^1H NMR Data for Compounds **6a-f** and **7a-b** (δ)

compd	C ₉ -Me	C ₁₀ -NH	C ₁₂ -H	C ₁₀ -H	aromatic protons
6a	2.07	6.42	5.46	5.61 (d) $J = 10.8$ Hz	7.20 (d, 1 H, $J = 3.6$ Hz), 6.63 (d, 1 H, $J = 3.6$ Hz)
6b	2.05	5.20	5.45	5.61 (d) $J = 10.8$ Hz	7.16 (m, 4 H)
6c	2.06	5.07	5.46	5.66 (d) $J = 10.8$ Hz	7.22 (m, 5 H)
6d	1.89	5.22	5.40	6.58 (d) $J = 10.8$ Hz	8.14 (m, 1 H), 7.46 (m, 1 H), 6.95 (d, 1 H, $J = 8.1$ Hz), 6.75 (m, 1 H)
6e	1.87	5.65	5.36	7.18 (d) $J = 10.8$ Hz	8.33 (d, 2 H, $J = 4.5$ Hz), 6.70 (t, 1 H, $J = 4.5$ Hz)
6f	1.87	5.24	5.39	6.53 (d) $J = 10.8$ Hz	8.18 (d, 1 H, $J = 1.8$ Hz), 7.66 (dd, 1 H, $J = 1.8$ and 9 Hz), 6.76 (d, 1 H, $J = 9$ Hz)
7a	2.28	5.79	5.49	5.54 (d) $J = 4.5$ Hz	7.19 (d, 1 H, $J = 3.6$ Hz), 6.58 (d, 1 H, $J = 3.6$ Hz)
7b	2.31	4.62	5.47	5.14 (d) $J = 5.4$ Hz	7.17 (m, 2 H), 6.52 (m, 2 H)

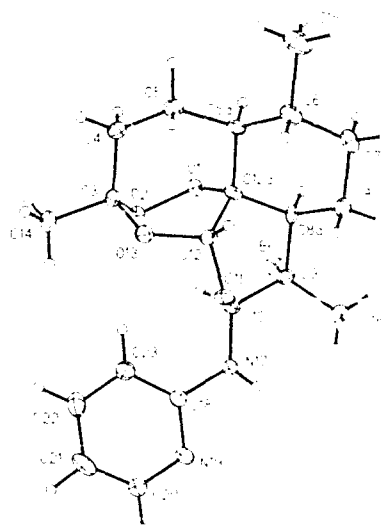
Treatment of **3** with bromine at low temperature gave a good yield of the corresponding dibromide (**4**). Inasmuch as two asymmetric carbon atoms are created during the bromination, four possible dibromide isomers are theoretically possible. However, **4** was found to be unstable at room temperature and, therefore, was used for reactions with amines without purification.

Condensation of dibromide **4** with amines was carried out in anhydrous dichloromethane at $<-10^\circ\text{C}$. Because this reaction involves the formation of 1 equiv of HBr, 2 equiv of amine were necessary for each equivalent of dibromide employed. Purification of the crude product was achieved through silica gel chromatography and yields ranged from 25–55% (Table I).

In contrast to aromatic amines, aliphatic amines such as benzylamine, butylamine, and *N,N*-diethylethylenediamine, gave no desired product under identical conditions. Instead, the major isolable product was 9,10-dehydrodihydroartemisinin (**3**) which was also found to be the minor product of reactions between aromatic amines and **4**.

Since two asymmetric carbon centers (C₉ and C₁₀) are created during the process of conversion from **3** to **6** and **7**, four stereoisomers are possible in the final products of each reaction. However, only two isomers were isolated in reactions when 3-fluoroaniline and 2-aminothiazole were used, and only one isomer was separated when aniline, 2-aminopyridine, and 2-aminopyrimidine were employed as reactants. In addition to **6d**, a byproduct was also isolated from the reaction mixture which contained 2-aminopyridine and was identified as 5-bromopyridine analogue **6f** by NMR (Table II). Compound **6f** presumably was derived from the bromination of the starting amine, 2-aminopyridine, or the product, **6d**. The source of the bromine atom was most likely from the oxidation of bromide anion by the hydroperoxide which is derived from the partial decomposition of the starting dibromide **4** during the reaction.

That there is little or no difference in chemical shift of C₁₂-H of all products suggests that compounds **6** and **7** have the same configuration at C₁₀ (Table II). This leaves the structural differences between **6** and **7** to occur at C₉. Notably, C₁₀-H of **6a-e** are deshielded by C₉-Br to cause a greater downfield shift than **7a-b**, whereas C₉-CH₃ of compounds **7a-b** resonates at lower field ($\Delta\delta$ 0.2–0.3 ppm) than compounds **6a-f**, due to the deshielding effect of the peroxide group. A molecular model shows that the methyl group and the peroxide function are in close proximity in compounds **7a-b**. These results suggest that the bromine in compounds **7a-b** is in the β configuration (i.e., on the same side as the C₁₂-H), whereas the bromine in compounds **6a-f** assumes an α configuration. Only one bromo group was displaced by an amino function when dibromide **4** was treated with amine at $<-10^\circ\text{C}$. This suggests that the C₁₀-Br and not the C₉-Br is displaced by amine. Since C₁₀-Br of **4** is attached to the same carbon as C₁₀-O, electron delocalization from oxygen will lead to formation of a reactive oxonium ion **5** which is then reacted with amines

**Figure 1.** Thermal ellipsoid plot drawing of **6d** at 20% probability level from experimental coordinates.

to give the observed products **6a-e** and **7a-b**. Steric hindrance of the α side of the intermediate **5** caused the preferential attack by the amine from β side which accounts for the observation that compound **6** is the major or the sole product of the condensation reactions. Like the ether formation of dihydroartemisinin with an alcohol under the catalysis of boron trifluoride etherate which gave mainly the β isomer and was also involved the oxonium ion as the intermediate,² the C₁₀-N configuration is also β in both products **6** and **7**.

A molecular model indicated that, due to steric hindrance by the surrounding functional groups, the rotation of the bulky heterocyclic or aromatic ring of the new compounds along the C₁₀ and N bond is restricted and, thus, only two conformations are possible for N-H, with the amine proton skewed between both protons at C₈ (α -H and β -H) or between the C₁₂-H and the β -C₈-H. The latter conformation gave larger J value than the former conformation due to larger dihedral angle between C₁₀-H and the N-H. The J value on C₁₀-H (Table II) suggested that compounds **6a-f** assume the latter conformation whereas compounds **7a-b** have the former conformation. The chemical shift and the coupling constant of C₁₀-H and C₁₀-NH of the final products were established by D₂O exchange technique.

The structure assignment of **6d** was confirmed by X-ray study. The results of the study are illustrated in Figure 1. The absolute configuration of **6d** (C_{5a}, C₉, and C₁₀ are *S* and C₃, C₁₂, C_{12a}, and C_{8a} are *R*) agrees with that found for artemisinin (**1**).¹⁵ With the exception that the brominated six-membered ring is more boat-shaped than

Table III. In Vitro Antimalarial Activity of Dihydroartemisinin Derivatives against *Plasmodium falciparum*

compd	IC ₅₀ , ng/mL	
	African clone (D-6)	Indochina clone (W-2)
6a	0.91	0.21
6b	0.16	<0.16
6c	3.42	0.63
6d	7.72	1.19
6e	25.96	5.56
1	0.56	0.61

chair-shaped, the overall conformation of the fused ring system in **6d** is similar to that found for both artemisinin and its derivatives such as artemether and dihydroartemisinin (**2a**).¹⁶ There is no hydrogen bonding in this structure with the closest N₁₇ intermolecular approach being N₁₇...O₁₃ at 3.21 Å. The closest intermolecular approach to the Br atom is a van der Waal's distance of 3.40 Å to a neighboring O₂ atom.

Results and Discussion

The new derivatives were tested in vitro against clones of human malaria, *P. falciparum* D-6 (Sierra Leone clone) and W-2 (Indochina clone). The former clone is a strain that is resistant to mefloquine and the latter, to chloroquine, pyrimethamine, sulfadoxine, and quinine.

The results (Table III) indicate that the new derivatives, like the parent agent **1**, are not cross-resistant with any of the antimalarial agents mentioned. As was observed with other water-soluble dihydroartemisinin derivatives, these new agents are more effective against W-2 than D-6. Compound **6b**, 3-fluoroaniline derivative, was the most active compound of the series, with the IC₅₀ (50% inhibitory concentration) ≤ 0.16 ng/mL making it several fold more potent than artemisinin. Compounds **6a** and **6c** showed activity comparable to artemisinin, whereas **6d** and **6e** are less active than the parent compound.

Despite the in vitro activities against *P. falciparum* observed with **6a-c**, no significant antimalarial activity against rodent malaria *P. berghei* in vivo was observed in any of the new compounds tested. No antimalarial testing was conducted on **7a-b** due to insufficient supply of these isomers.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr disks on a Nicolet 20SXB FT-IR spectrometer. NMR spectra were determined on a JEOL FX90Q spectrometer with Me₄Si as an internal standard. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and the results are within 0.4% of the theoretical values, except where noted.

Synthesis of 9,10-Dehydrodihydroartemisinin (3). Dihydroartemisinin (**2a**)² (5 g, 17.6 mmol) was dissolved in 30 mL of anhydrous Et₂O and cooled to 0–5 °C. Boron trifluoride etherate (5 mL in 50 mL of anhydrous Et₂O) was added dropwise to the solution with stirring. The solution was allowed to stand at room temperature overnight, washed first with aqueous NaHCO₃, followed by saturated aqueous NaCl solution, and dried over MgSO₄. The solvent was evaporated to dryness under the reduced pressure. The residue was chromatographed on a silica gel column using hexane/EtOAc (3:1 v/v) as eluent to give 4.0 g (85%) of colorless crystals of **3**: mp 96–98 °C (lit.² mp 95–97 °C), R_f = 0.74 (silica gel, hexane/EtOAc 3:1 v/v).

Bromination of 9,10-Dehydrodihydroartemisinin (3). To the solution of compound **3** (100 mg, 0.38 mmol) in 5 mL of anhydrous CCl₄ was added dropwise with stirring a 5% solution

of bromine in anhydrous CCl₄ until the mixture turned pale orange in color. After the mixture was stirred for an additional hour, the solvent was removed under reduced pressure at <40 °C and was used in the subsequent step without further purification. The bromo products (**4**) gradually turned to a black tar on standing at room temperature.

General Procedure for the Condensation of 4 with Amines. An amine (2 mmol) was dissolved in 10 mL of anhydrous CH₂Cl₂ and cooled to –10 °C with a ice/salt bath. The amine solution was added dibromo compound **4** (1 mmol) in 5 mL of CH₂Cl₂ dropwise with stirring. After the addition was completed, the solution was allowed to stand at room temperature overnight. The reaction mixture was washed with saturated aqueous NaHCO₃ solution, dried over MgSO₄, and evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography using hexane and EtOAc mixed solvent as eluent to give the desired final products (Table I).

X-ray Crystallographic Data for 6d. C₂₀H₂₇N₃O₄Br, molecular weight = 546.48, clear colorless data crystal (0.18 × 0.12 × 0.48 mm), orthorhombic, space group P2₁2₁2₁, a = 10.118 (3) Å, b = 10.663 (4) Å, c = 18.461 (5) Å, d_{calc} = 1.82 mg mm⁻³, Z = 4, μ = 2.1 mm⁻¹, 2620 independent reflections were measured out of 29,000, 2θ_{max} = 45.0° with a Nicolet R3m/V diffractometer using Mo Kα radiation (λ = 0.70169 Å) with a graphite monochromator in the incident beam. The data were collected at room temperature by using the θ/2θ scan technique with a variable scan rate ranging from 10°/min minimum to 30°/min maximum, depending upon the intensity of a reflection. Data were corrected for Lorentz and polarization effects, and an empirical absorption correction was applied (maximum and minimum transmission factors were 0.786 and 0.695, respectively). The structure was solved by direct methods as implemented by the SHELXTL system of programs.¹⁷ Full-matrix least-squares refinement on 249 parameters (coordinates and anisotropic thermal parameters for nonhydrogen atoms; hydrogen atoms originally placed at calculated positions and then allowed to ride on covalently bonded atoms, that is, C–H distances set 0.96 Å and coordinate shifts of attached C atom also applied to H atom, C–C–H angles set as close to idealized values as possible) used the 2270 reflections for which |F_o| > 3σ(F_o). The absolute configuration of **6d** was determined from the difference in R factors on the basis of the anomalous scattering of the Br atom by using Friedel's pairs as suggested by Rogers.¹⁸ The final R factors for the configuration shown in Figure 1 were R = 0.043 and R_w = 0.042 (for the "wrong" hand these values were R = 0.070 and R_w = 0.075). The goodness of fit parameter was 1.4, and the final difference map was featureless.

Biology. (a) In Vitro Antimalarial Studies. The in vitro assays were conducted with use of the semiautomated microdilution technique of Desjardins et al.¹⁹ as modified by Milhous et al.²⁰ Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates obtained by the Centers for Disease Control, Atlanta, GA in 1980 and 1982, respectively. The patients had acquired infections either in Vietnam or Sierra Leone. The Indochina clone is resistant to the antimalarials chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone, is resistant to mefloquine but susceptible to chloroquine, quinine, sulfadoxine, and pyrimethamine. Test compounds were initially dissolved in DMSO and 70% ethanol and diluted in RPMI 1640 culture medium with 10% human plasma to 400-fold. Drugs were subsequently further diluted by using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of 1.56–100 × 10⁻⁸ molar. Parasite inocula (at 0.5% parasitemia and a 1% hematocrit) were incubated for 24 h and added to equimolar concentrations of each test compound prior to the addition of ³H-hypoxanthine. After a further incubation

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of 18 h, particulate matter was harvested from each microtiter well with use of an automated cell harvester (Skatron, Inc., Sterling, VA). Uptake of ^3H -hypoxanthine was measured by using a scintillation spectrophotometer (Model LS3801, Beckman Instruments, Irvine, CA). Concentration-response data were analyzed by nonlinear regression and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated.

(b) In Vivo Antimalarial Studies. The suppressive blood schizonticidal and curative activities of these new compounds were measured in a test where mice were infected with 5.98×10^5 *P. berghei* parasitized cells intraperitoneally on day 0. Test compounds were dissolved in peanut oil and were administered subcutaneously once a day for three consecutive days commencing on day 3. The dose levels of compounds given were 640, 160, and 40 mg/kg per day. Blood films were taken on days 6, 13, and 20. Blood schizonticidal activity was determined by monitoring blood films for the appearance of parasites and for extended survival times compared to infected untreated controls. Mice surviving 60 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds was considered active when the survival time of the treated mice

was greater than twice the control mice, i.e., 12-14 days.

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Registry No. 1, 63968-64-9; 29, 71939-50-9; 3, 82596-30-3; 4, 127971-91-9; 69, 127971-92-0; 66, 127997-42-6; 6c, 127997-43-7; 6d, 127971-93-1; 6e, 127971-94-2; 6f, 127971-95-3; a, 128050-94-9; 7b, 128052-60-8; 3- $\text{FC}_6\text{H}_4\text{NH}_2$, 372-19-0; $\text{C}_6\text{H}_5\text{NH}_2$, 62-53-3; 2-aminothiazole, 96-50-4; 2-aminopyridine, 504-29-0; 2-aminopyrimidine, 109-12-0.

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates for compound 6d (5 pages). Ordering information is given on any current masthead page. Tables of atomic coordinates and bond lengths and angles have been deposited with the Crystallographic Data Centre, Cambridge University Chemical Laboratory, Cambridge CB2 1EW, England.



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